REMARKS

The Examiner is thanked for the Office Action of July 29, 2003, rejecting pending claims 1 and 2 under 35 USC §112 and §103. The Applicant respectfully asserts that the following discussion below fully addresses and overcomes all the issues raised by the Examiner in the Office Action to put the Application in condition for Allowance.

Rejection under 35 USC 112

As to the rejection under 35 U.S.C. §112,"WHb-DAAO" in claims 1 and 2 has been corrected with the expansion of the expression "*Vitreoscilla* hemoglobin-D-amino acid oxidase" and addresses the issue raised by the Examiner. The scope of the claim has not been changed, but the invention has been clarified.

Rejection under 35 USC 103 under Khang I (1996) view of Huston et al.

The Examiner rejected claims 1 and 2 as unpatentable over Khang I (1996) in view of Huston with Frey teaching knowledge of the skilled artisan. The Applicant respectfully that the rejection under 35 U.S.C. §103 (a) was improper, because the presently claimed invention differs from the combination of the cited references (publications by Huston et al., Khang I et al., and Frey et al.), and Frey does not provide the requisite knowledge of the skilled artisan. A summary of the cited references is as follows:

Khang I (1996) teaches that a recombinant *Vitreoscilla* hemoglobin and the recombinant D-amino acid oxidase are separately expressed under different promoters, purified, mixed and then co-immobilized. Thus in Khang I, *Vitreoscilla* and D-amino acid oxidase are not expressed as a single polypeptide, but separately expressed, purified, mixed and co-immobilized. Therefore, in Khang I, the two proteins were mixed simply by fixing on a matrix.

Huston et al. (U.S. Patent 5,013,653) teaches that in order to facilitate isolation and purification of a target protein, a polypeptide is fused with the target. *This patent teaches a method of preparing a new fusion protein not by fusing two whole proteins with different functions, but provides a target protein fused with a short peptide by employing an oligonucleotide.

Frey et. al teaches that *Vitreoscilla* hemoglobin is expressed in a state fused with reductase in *E. coli.* where the fusion protein improves the bacterial growth rate and increases expression of β-lactamase even under oxygen starvation. *Vitreoscilla* hemoglobin is linked to reductase, not oxidase, by employing an artificial linker peptide. The VHb-reductase fusion enzyme is not used as an immobilized enzyme after purification, but used only for improving the energy level in *E. coli.* The Examiner refers to Frey to detail a process of applying VHb in *E. coli* to enhance protein levels as allegedly well known to the skilled artisan. Furthermore, the Examiner states that Frey also portrays the knowledge of the skilled artisan by stating that VHb is introduced to E. coli to improve oxygen utilization by the cells.

The presently claimed invention discloses that *Vitreoscilla* hemoglobin is fused with D-amino acid oxidase. Then the single fusion protein is purified and used as an industrial enzyme in the bio-conversion of cephalosporin C. *Vitreoscilla* hemoglobin is linked to D-amino acid oxidase, not by employing an oligonucleotide or a linker peptide, but by PCR, resulting in production of a single polypeptide. The artificial VHb-oxidase fusion enzyme is expressed in *E. coli*, purified after cell lysis, and developed for use as an industrial enzyme.

It has been reported that the intracellular expression of *Vitreoscilla hemoglobin* (VHb) increases production yield of proteins or physiologically active materials. This phenomenon is believed to originate from the fact that, by action of VHb with oxygen-binding ability, the increased proton (H) pumping level in the electron transport chain results in an increase of ATP synthesis and thus overall bacterial activity, leading to the enhanced production of proteins or physiologically active materials. However, until now, VHb has been used only for improving

microbial activity via expression in microorganisms. In support of this, in the publication by **Frey et al.** (2000), and used by the Examiner to show the knowledge of the skilled artisan, the VHb-reductase fusion protein is also used only in the bacteria. VHb was fused with NAn+-requiring reductase, not oxygen-requiring oxidase, and the fusion protein is used only for indirectly improving the activity of *E. coli*. The present Application excepted, the Applicant's believe that there is no report associated with the case where the VHb fused-with oxidase is expressed as a single polypeptide, purified and developed as a biocatalytic enzyme. Thus, applying the teachings of Frey as knowledge by the skilled artisan that address the problems in the technical field that solved by the present invention is not appropriate in a 103 rejection. *See* **MPEP 2141.03**.

Certain protein fusion techniques are known to those skilled in the art. However, when a wild-type protein is fused with a peptide of a different protein, it often undergoes conformational change and thus losses its function or stability. In addition, it requires a great deal of experimental trial and error to develop a fusion protein while maintaining biological functions of both different proteins. For these reasons, most of enzymes used in the bio-industry are wild-type, and not artificially fused with a different protein as claimed by the present invention.

Huston et al. describes a method of preparing a fusion protein. However, this method is relatively simple since a target protein is linked to a short peptide by employing an oligonucleotide. In addition, the short peptide is used to facilitate purification of the target protein, and so, cleaved out from the fusion protein after purification in order to recover the natural structure of the target protein. This does not apply to the presently claimed invention, nor would have been combined with Khang I (1996) to render the presently claimed invention obvious.

The background or knowledge of the skilled artisan reference, **Frey et al** teaches that (2000), 5-7 amino acid peptides are used as a linker to fuse VHb with reductase (not oxidase), thereby maintaining the active structures of both proteins.

Contrastingly, in the presently claimed invention, such an oligonucleotide or a linker peptide is not used. Instead, the VHb gene is directly linked to the D-amino acid oxidase gene by PCR (Polymerase Chain Reaction) in order to maintain the biological functions of both proteins. Therefore, the present invention is completely different from the two cited references and the background reference regarding the knowledge of the skilled artisan, and would not be taught or suggested by the combination.

In summary, the characteristic differences of the present invention from the two cited references and the background reference are as follows:

(1) VHb is not used to indirectly improve bacterial activity or production of a recombinant protein;

(2) Fusion of VHb with D-amino acid oxidase is achieved without use of a short peptide or linker peptide;

and

(3) Neither VHb nor D-amino acid oxidase is separately used fox-co-immobilization on a matrix. A single polypeptide of VHb-D-amino acid oxidase is used for immobilization on a matrix.

The Examiner states that it is known (Frey) that VHb has an ability to bind molecular oxygen and D-amino acid oxidase requires molecular oxygen in the catalytic reaction. However, the novelty of the presently claimed invention is disclosed by the fact that D-amino acid oxidase can utilize molecular oxygen carried by VHb directly on a single polypeptide by a suitable protein fusion technique.

Therefore, the claimed invention of the artificial fusion enzyme of VHb and D-amino acid oxidase, which is prepared by linking the gene of VHb to the gene of D-amino acid oxidase by PCR to express the two proteins as a single polypeptide, and

the method of preparing the same involve significant inventive processes and are clearly not obvious over a combination of the cited references.

CONCLUSION

For at least the above-stated reasons, the Applicants assert that the presently claimed invention is not obvious in view of the references cited by the Examiner, and that claims 1 and 2 are now in condition for Allowance. Hence, a Notice of Allowance is earnestly solicited. Should the Examiner believe that a telephone or in-person conference would help to resolve any remaining issues and/or expedite the prosecution of this application, they are invited to contact the Applicant's counsel at the contacts listed below.

Respectfully Submitted,
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